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REVIEW

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Human Pluripotent Stem Cell-Based Approaches for Myocardial Repair: From the Electrophysiological Perspective

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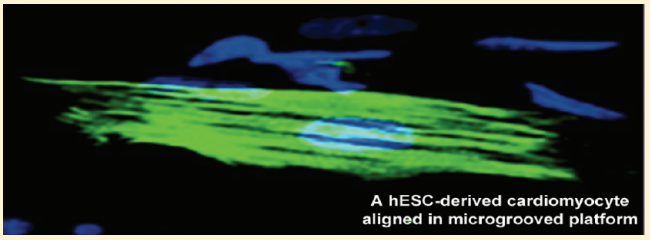
ABSTRACT: Heart diseases are a leading cause of mortality worldwide. Terminally differentiated adult cardiomyocytes (CMs) lack the innate ability to regenerate. Their malfunction or significant loss can lead to conditions from cardiac arrhythmias to heart failure. For myocardial repair, cell- and gene-based therapies offer promising alternatives to donor organ transplantation. Human embryonic stem cells (hESCs) can self-renew while maintaining their pluripotency. Direct reprogramming of adult somatic cells to become pluripotent hES-like cells (also known as induced pluripotent stem cells or iPSCs) has been achieved. Both hESCs and iPSCs have been successfully differentiated into genuine human CMs. In this review, we describe our current knowledge of the structure−function properties of hESC/iPSC-CMs, with an emphasis on their electrophysiology and Ca^{2+} handling, along with the hurdles faced and potential solutions for translating into clinical and other applications (e.g., disease modeling, cardiotoxicity and drug screening).



KEYWORDS: human embryonic stem cells, induced pluripotent stem cells, heart, electrophysiology, cardiomyocytes

HEART DISEASE AND CELL-BASED THERAPY

Circulation requires the highly coordinated efforts of chamber-specific pacemaker, atrial and ventricular cardiomyocytes (CMs), which differ in their morphological, structural and functional properties. Normal rhythms originate in the sinoatrial node (SAN), a specialized cardiac tissue consisting of only a few thousand pacemaker cells. In the process of pacing, the SAN spontaneously generates rhythmic action potentials (AP) which subsequently propagate to induce coordinated muscle contractions of the atria and ventricles for effective blood pumping. Since terminally differentiated adult CMs lack the ability to regenerate, their malfunction due to aging or significant loss under pathophysiological conditions (e.g., myocardial infarction) can lead to consequences from arrhythmias (such as SAN dysfunction that necessitates electronic pacemaker implantation) to heart failure (primarily a disease of the ventricle). For patients with end-stage heart failure, heart transplantation remains the last resort, but this option is limited by the number of donor organs available. As such, cell replacement therapy presents a laudable alternative. Various cardiac and noncardiac lineages have been suggested as potential cell sources. Transplantable human CMs (e.g., human fetal CMs) appear to be the most relevant, but substantial practical and ethical limitations exist. Therefore, noncardiac cells such as skeletal muscle myoblasts (SkM), mesenchymal stem cells and smooth muscle cells have been sought as potentially viable alternatives. However, the noncardiac identity of these cell sources presented major limitations. For instance, it is now established that although bone marrow stem cells improve cardiac functions of ischemic patients by promoting angiogenesis, they lack the capacity to transdifferentiate into cardiac muscle for myocardiogenesis.[1,2] Due to the absence of conduction via gap junctions, the lack of electrical integration of SkM after their autologous transplantation into the myocardium has been shown to underlie the generation of malignant ventricular arrhythmias, which led to the premature termination of their clinical trials.[3,4] In this review, we will focus our discussion on human pluripotent stem cells.

HUMAN EMBRYONIC STEM CELLS (hESC) AND INDUCED PLURIPOTENT STEM CELLS (iPSC)

Human embryonic stem cells, isolated from the inner cell mass of blastocyst, can self-renew while maintaining their pluripotency to differentiate into all cell types,[5] including CMs.[6,7] Therefore, in principle, hESCs can serve as an unlimited *ex vivo* source of CMs for cell-based heart therapies. Indeed, hESC-derived CMs (hESC-CMs) have been reported to partially restore impaired cardiac functions in several animal models of myocardial infarction.[8,9] However, a range of ethical and technical hurdles (e.g., immune rejection of the transplanted grafts) have vastly limited their translation into clinical applications. Direct reprogramming of adult somatic cells to become pluripotent hES-like cells (also known as induced pluripotent stem cells or iPSCs) has

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been achieved by Yamanaka[10] and Thomson,[11] potentially eliminating both ethical concern and the issue of immune rejection. Forced expression of four pluripotency genes (Oct3/4, Sox2, c-Myc, and Klf4 or Oct3/4, Sox2, Nanog, and Lin28)[10−12] suffices to reprogram mouse and human fibroblasts into iPSCs. Recent studies have further demonstrated the successful use of fewer pluripotency factors[13−15] and nonviral methods (e.g., with synthetic modified RNA[16]) to reprogram somatic cells into patient-specific iPSCs. Although concerns such as induced somatic coding mutations[17] have yet to be fully addressed, iPSCs largely resemble hESCs in terms of their pluripotency, surface markers, morphology, proliferation, feeder dependence, global transcriptomic profile and epigenetic status, promoter activities, telomerase activities, and *in vivo* teratoma formation.[10,11] Importantly, iPSCs can likewise be differentiated into CMs.[18] Adopting a similar reprogramming approach, more recent studies have reported the successful direct conversion of fibroblasts into cardiomyocytes,[19] although their functionality and the underlying mechanisms for such cell fate conversion require further investigations and scrutinity (see also review by Xu et al.[20]).

CARDIAC DIFFERENTIATION

CMs originate from the mesodermal germ layer. During the course of gastrulation, cardiac progenitors migrate through the node region and primitive streak to form the cardiac crescent.[21−23] At this stage, CMs become specified, along with the expression of various cardiac transcription factors. Fetal CMs continue to proliferate until they terminally exit the cell cycle a few days after birth. Further growth is accomplished via physiological hypertrophy by increasing the size rather than the number of CMs.[24,25] Subsequent development of CMs also involves the structural and functional maturation of their electrophysiological, Ca^{2+}-handling and contractile properties. Taken collectively, the formation of the adult heart is a complex developmental event, involving the orchestrated interplay of numerous biological factors and processes.

Early studies have demonstrated that murine (m) ESCs can spontaneously differentiate into CMs when they aggregate in suspension to form 3-dimensional embryoid bodies (EBs).[26] Indeed, the developmental changes of mESC-derived CMs differentiated *in vitro* mimic those seen in early myocardial development *in vivo*: pacemaker-, atrial- and ventricular-like derivatives appear and predominate at early, intermediate and late stages of cardiac differentiation, respectively, although a heterogeneous population of all three CM types is almost always seen in mouse EBs. Similar to the murine counterpart, hESC (and iPSC) can be spontaneously differentiated into a composite of specialized cells types including CMs.[6,7,27,28] Cardiac differentiation can also be enhanced by coculture with visceral endoderm-like cells[29] or promoted by stage-specific induction by specific combinations of growth and transcription factors.[30,31] Other approaches such as the use of different extracellular matrices, serum[32] and insulin elimination[33] have also been pursued. Keller and colleagues (2008) have reported the specification of human tripotent $KDR^{low}/c\text{-}kit^{neg}$ cardiovascular progenitors (CP) into CMs.[34] Driven mesodermal differentiation results in generation of a $KDR^{low}/c\text{-}kit^{neg}$ CP population which defines one of the earliest stages of human cardiac development. CP-containing cardiogenic EBs or "cardiospheres" derived from hESCs can efficiently differentiate into CMs *in vitro* with >50% yield, as gauged by the proportion of cells that express troponin T. Further modifications of this protocol enable the induction of a large CP population, allowing the generation of highly enriched CMs (with yields orders of magnitude higher than that of the conventional method of EB formation) even without the need for cell sorting. Therefore, hESC/iPSCs can in principle provide an unlimited *ex vivo* source of CMs for disease modeling and cell-based heart therapies. However, terminal differentiation and derivation of specific CM subtypes have not yet been accomplished but are actively pursued by many laboratories, including our own. Various purification methods including Percoll gradient centrifugation,[7] optical signatures,[35] and genetic selection based on the expression of a reporter protein under the transcriptional control of a cardiac-restricted promoter (e.g., α-MHC,[36] MLC2v[37,38]) have been developed to generate purer preparations of CMs.

ELECTROPHYSIOLOGY AND Ca^{2+}-HANDLING AS FUNDAMENTAL PROPERTIES OF CMs

Upon *electrical* stimulation of a ventricular muscle CM by pacemaking signals, voltage-gated Na (I_{Nav}) channels open to initiate an AP, during which Ca^{2+} ions enter into the cytosol through sarcolemmal L-type Ca^{2+} ($I_{Ca,L}$) channels, triggering the release of Ca^{2+} from the intracellular Ca^{2+} stores (also known as sarcoplasmic reticulum, SR) via ryanodine receptors (RyR). This so-called Ca^{2+}-induced Ca^{2+}-release (CICR)[39] escalates the cytosolic Ca^{2+} ($[Ca^{2+}]_i$) to activate the contractile apparatus for *mechanical* contraction. For relaxation, elevated $[Ca^{2+}]_i$ gets pumped back into the SR by the sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) and extruded by the Na^+-Ca^{2+} exchanger (NCX) to return to the resting $[Ca^{2+}]_i$ level. Such a rise and subsequent decay of $[Ca^{2+}]_i$ are known as *Ca^{2+} transient*. Both the contractile force and frequency of CMs depend on the Ca^{2+} transient amplitude and kinetics. Given the central importance of CICR in excitation−contraction (EC) coupling, *proper electrophysiological and Ca^{2+} handling properties are crucial for CMs to mechanically function.* Abnormal electrophysiology and Ca^{2+} handling, as in heart failure, can be arrhythmogenic.[39]

In mature ventricular CMs (VCMs), CICR is facilitated by the presence of t-tubules, invaginations in the sarcolemmal membrane that concentrates $I_{Ca,L}$ channels and brings them spatially closer to RyRs residing on the SR membrane located deeper in the cytoplasm.[40,41] By physically minimizing the diffusion distance, RyRs in CMs can participate in CICR without a lag. The result is a synchronized, faster, and greater transient $[Ca^{2+}]_i$ increase from the periphery to the center, creating a uniform Ca^{2+} wavefront across the transverse section with simultaneous recruitment of all SR. Fast and synchronized activation of RyRs translates into a greater Ca^{2+} transient amplitude, recruitment of more actin−myosin cross-bridge cycling, and generation of greater contractile force. Such a uniform Ca^{2+} wave starkly contrasts with the U-shaped Ca^{2+} wave propagation from the periphery to the center in a detubulated ventricular or atrial CM (that lacks t-tubules) such as those from failing hearts.[40] The U-shaped waves result from a time delay that is proportional to the diffusion distance squared in recruiting the Ca^{2+} stores at the cell center.[42]

STRUCTURAL AND FUNCTIONAL PROPERTIES OF HUMAN ESC- AND iPSC-derived CMs

Human ESC-CMs have been structurally and functionally characterized by us and several laboratories. Human ESC-CMs express cardiac-specific transcription factors and structural proteins.[6,7,27−29] Although all hESC lines are by definition

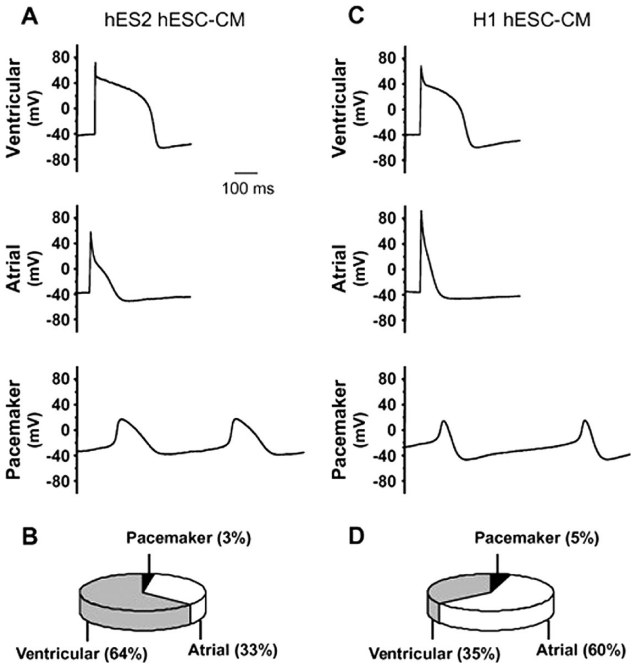

Figure 1. (A, C) Action potentials and (B, D) pie graphs showing the % distribution of chamber-specific CMs from HES2 and H1, respectively. Reprinted with permission from ref 43. Copyright 2008 Elsevier Inc.

pluripotent, we have reported that different lines have distinct cardiogenic potentials to become early ventricular-, atrial- and pacemaker-like derivatives as gauged by their signature AP profiles. For instance, HES2 cells have a higher likelihood than H1 cells of differentiating into ventricular-like hESC-CMs[43] (Figure 1). Using the same methods for hESCs, human iPSCs can likewise differentiate into CMs with nodal-, atrial-, or ventricular-like electrophysiological phenotypes.[18] The derived CMs also expressed cardiac-specific transcription factors and structural proteins.[44] Positive and negative chronotropic responses can be induced by isoproterenol and carbamylcholine, respectively.[44] In the two subsections that follow, we will focus our discussion on the Ca^{2+}-handling and electrophysiological properties of hESC-CMs.

Ca^{2+}-Handling of hESC/iPSC-CMs Is Functional but Immature. Dolnikov and colleagues (2006) were the first to study the Ca^{2+}-handling properties of hESC-CMs in detail.[45] They reported that Ca^{2+} transients recorded from spontaneously beating or electrically stimulated hESC-CMs respond to neither caffeine nor ryanodine; hESC-CMs recorded as beating clusters also displayed a negative force−frequency relationship that is different from that of adult CMs. Based on these observations, the authors concluded that hESC-CMs are immature and do not express functional SRs, and that their contractions result from trans-sarcolemmal Ca^{2+} influx (rather than Ca^{2+} release from the SR). Our laboratory compared Ca^{2+} transients from hESC-CMs and human fetal left ventricular (LV) CMs (16−18 weeks), and demonstrated the presence of functional SRs even in hESC-CMs that were younger (Figure 2)[46] (18−24 vs 55 day old postdifferentiation of Dolnikov et al). Upon electrical stimulation, hESC-CMs generated Ca^{2+} transients similar to fetal LV-CMs. However, caffeine-induced Ca^{2+} release was observed in 65% of fetal LV-CMs but only ∼40% of H1- and HES2-CMs. Ryanodine significantly reduced the electrically evoked Ca^{2+} transient amplitudes and slowed the upstroke of caffeine-responsive HES2- and H1-CMs but not caffeine-insensitive cells; thapsigargin, a SERCA inhibitor, similarly reduced the amplitude and slowed the decay of only caffeine-responsive HES2- and H1-CMs.[46] The discrepancy between our findings and those of Dolnikov et al. can be largely attributed to the newly identified caffeine-responsive population.

Human ESC-CMs and adult CMs display different expression patterns of Ca^{2+} handling proteins. For instance, NCX protein expression is higher in hESC-CMs and fetal LV-CMs compared to adult LV-CMs. The same trend is also observed at the functional level.[38] By contrast, SERCA2a is highest in adult LV-CMs, although it is also substantially and comparably expressed in hESC- and fetal LV-CMs. RyRs is robustly expressed in hESC-CMs and fetal LV-CMs but in a disorganized manner compared to adult LV-CMs. The regulatory proteins junctin, triadin, and calsequestrin (CSQ) are expressed in adult LV-CMs but are completely absent in hESC-CMs.[46] Similarly, phospholamban (PLN) is also not expressed. Additionally, hESC-CMs do not display t-tubules (Figure 3)[47] that facilitate CICR. Table 1 summarizes some Ca^{2+}-handling properties of hESC-, fetal and adult CMs.

While Ca^{2+}-handling proteins including $I_{Ca,L}$ channels, RyR, SERCA and NCX together orchestrate the Ca^{2+} homeostasis of CMs, regulatory proteins such as triadin, junction and calsequetrin (CSQ), which are coupled to RyRs at the luminal SR,[48] also play a significant role in mediating CICR. For instance, CSQ2 is the most abundant, high-capacity but low-infinity Ca^{2+}-binding protein in the SR. The cardiac isoform can store up to 20 mM Ca^{2+} while buffering the free SR $[Ca^{2+}]$ at ∼1 mM,[49] allowing repetitive muscle contractions without run-down. CSQ2 also coordinates the rates of SR Ca^{2+} release and loading by modulating RyR activities, serving as an important determinant of the SR load, which in turn will affect the extents and kinetics of CICR. Our laboratory has demonstrated that forced expression of CSQ, which is otherwise completely absent in hESC-CMs, leads to functional improvements of Ca^{2+} transient parameters as a sign of maturation (Figure 4).[50] However, CSQ-matured hESC-CMs continued to have immature electrical properties. Genetic suppression of NCX did not lead to a more mature Ca^{2+}-handling phenotype suggesting transgene-specificity (H. M. Yeung and R. A. Li, unpublished data).

Electrophysiological Properties of hESC/iPSC-CMs. Adult left ventricular (LV) CMs are normally electrically silent-yet-excitable upon stimulation. The cardiac AP is a result of multiple ion channels and Ca^{2+}-handling proteins interacting in a highly coordinated manner at the single- and multicellular levels. Assessing the AP generation and profiles of hESC/iPSC-CMs provides us with valuable information regarding their specific identity and functionality. He et al. (2003) were the first to study the electrophysiological properties of hESC-CMs.[27] They characterized the APs from beating EB outgrowths cultured for 40 to 95 days, with depolarized maximum diastolic potentials (MDP) and slowed APs that are typical of immature embryonic CMs. Unlike the adult counterparts, our own data further showed that the majority of ventricular hESC-CMs fire spontaneously, exhibiting a high degree of automaticity. The remaining quiescent cells could elicit single APs upon stimulation, indicating that their excitability is intact. However, the electrical properties of hESC-CMs are in general immature, akin to arrhythmogenic, failing adult ventricular CMs: prominent "phase 4-like" depolarization, a known substrate for delayed after depolarization (DAD) and significantly depolarized resting membrane potentials (RMPs). Facilitated maturation by various approaches is one of the major objectives of current research. Building upon our series of cardiac

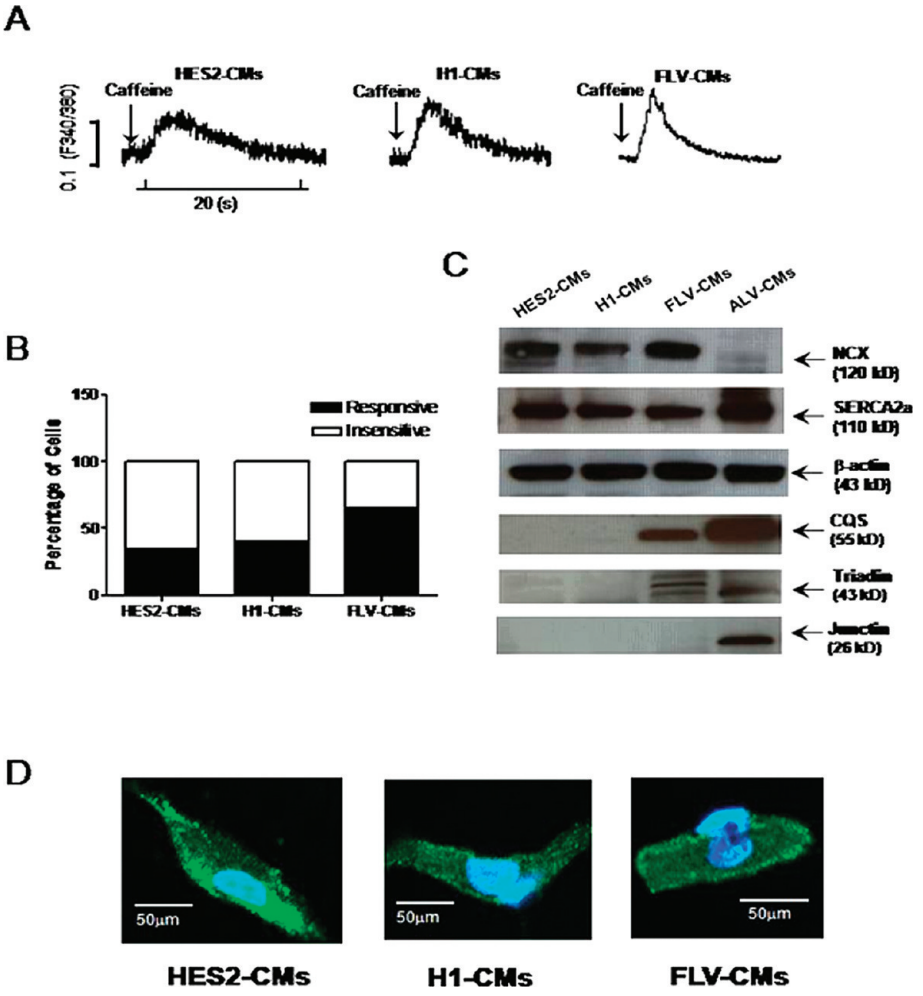

Figure 2. (A) Representative tracings of caffeine-induced Ca^{2+} transients of HES2-, H1-, and FLV-CMs. (B) % of caffeine-responsive and -insensitive cells. (C) Expression of various Ca^{2+} handling proteins. β-Actin was used as the loading control. (D) Immunostaining of RyRs. Reprinted with permission from ref 46. Copyright 2007 AlphaMed Press.

automaticity studies,[51−55] we identified the lack of Kir2.1-encoded I_{K1} in hESC-CMs as the primary mechanistic contributor to the immature pro-arrhythmic electrophysiological properties observed. Forced Kir2.1 expression alone sufficed to render the electrical phenotype indistinguishable from that of primary adult ventricular cells. We are currently developing a nongenetic, nonpharmacologic method for reproducibly driving global maturation, by targeting the microenvironmental niches and other noncell autonomous means.

Taken collectively, a number of major issues need to be addressed before hESC/iPSC-CMs can be used for clinical applications. In sum, hESC-CMs have immature Ca^{2+}-handling,[38,46,50] with an attenuated transient and heart failure-like U-shaped Ca^{2+} propagation wavefront due to the lack of t-tubule,[47] as well as immature electrical properties with pro-arrhythmic potentials.[51,56−58] Their physical size is typically ∼10 times smaller than adult CMs. Structurally, they lack the ordered organization at the sub-, single- and multicellular levels.[59] Furthermore, even with directed cardiac differentiation, the derived populations are always highly heterogeneous by consisting of a mixture of pacemaker, atrial and ventricular derivatives. However, unlike the hematopoietic and neuronal lineages, no convenient cardiac/chamber-specific surface markers have been identified for robust purification.[35] Recently, a nongenetic method has been developed for sorting pluripotent stem cell-derived CMs using a dye that labels mitochondria,[60] but atrial and ventricular muscle derivatives still cannot be readily distinguished. Importantly, the long-term safety and functional efficacy of hESC/iPSC-CMs are uncertain, given their reported poor graft survival *in vivo*[61] and our lack of understanding of their immunobiology.[62−65] Indeed, a recent study shows that iPSCs reprogrammed from autologous somatic cells display significant immunogenicity and can induce T-cell-dependent immune responses in syngeneic recipients, challenging the assumption of patient-specific immunocompatibility.[66] Whether the same applies to iPSC-CMs remains unknown. Despite these hurdles, with a better understanding of the basic biology of hESC/iPSC-CMs, as outlined in the select examples given above for Ca^{2+}-handling and electrophysiology, there is every reason to believe that more significant and robust advances can be achieved in the foreseeable future.

■ TISSUE ENGINEERING: PHYSICAL ALIGNMENT OF hESC-CMs AND ENGINEERED CARDIAC TISSUE CONSTRUCTS

The ventricular myocardium is a highly complex structure consisting of aligned, connected CMs, stromal cells and a

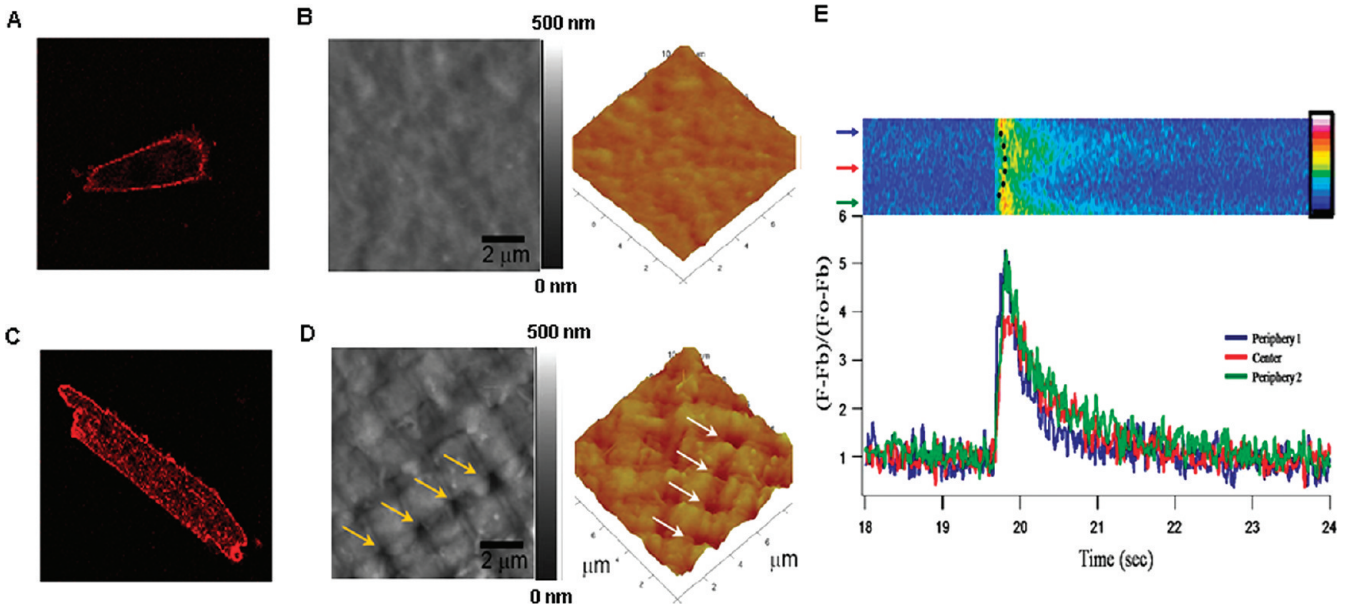

Figure 3. T-tubule imaging of a hESC-CM and a mature ventricular CM. Di-8-ANEPPS confocal microscopic images of a hESC-CM (A) did not show intracellular fluorescent spots like those in an adult guinea pig ventricular CM (C) suggesting the absence of t-tubules. The absence of t-tubules in ESC-CMs was further confirmed by atomic force microscopy (AFM) imaging of an adult ventricular cardiomyocyte (D) showing regularly spaced pores in the sarcolemma that coincide with the Z-lines, while hESC-CM (B) surface showed comparatively smoother topology with no presence of invaginations that are indicative of t-tubules. (E) Electrically induced Ca^{2+} transient in hESC-CMs. Top: Time progression linescans of pseudocolored transient increase in intracellular Ca^{2+} across the midplane of a hESC-CM showed a U-shaped wavefront. Bottom: Quantified Ca^{2+} transient of linescans of the top panel. Reprinted with permission from ref 47. Copyright 2009 Mary Ann Liebert, Inc.

Table 1. Table Summary of Differences[a]

		LVCMs	
	hESC-CMs	fetal	adult
Expression Levels of Ca^{2+}-Handling Proteins			
RyR	++	++	++++
SERCA	+++	+++	++++
phospholamban	−	++	++++
CSQ/Tdn/Jtn	−	+	++++
calreticulin	++++	++++	+
NCX	+++	++++	+
Ca^{2+} Transient Properties			
basal $[Ca^{2+}]_i$	++	+++	++++
amplitude	++	++	++++
decay	++	++	++++
upstroke	++	++	++++

[a] H1- and HES2-CMs have comparable trends and are collectively referred to as hESC-CMs.

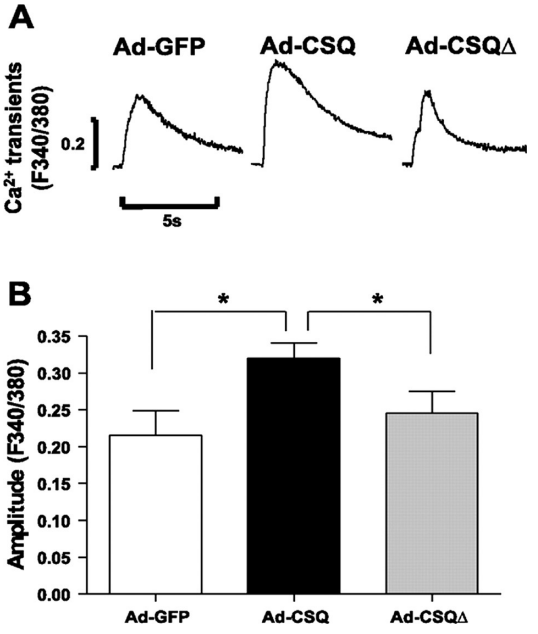

Figure 4. Effect of CSQ overexpression on hESC-CMs. (A) Representative electrically induced Ca^{2+} transient tracings for Ad-GFP (n = 12) and Ad-CSQ (n = 29) transduced hESC-CMs. (B) Bar graphs of amplitude. *P < 0.05, **P < 0.01 Reprinted with permission from ref 50. Copyright 2009 American Physiological Society.

vascular network systematically embedded in a mesh of extracellular matrix. Although the successful development of directed cardiac differentiation has made possible the derivation of hESC-CMs with high yields, hESC-CMs differentiated *in vitro* lack the subcellular organization and higher order structural 2- or 3-dimensionality seen in adult heart. Therefore, there is a need to apply tissue engineering techniques to put hESC-CMs together to better mimic the native heart tissues for better safety and efficacy. Indeed, when grown on a microgrooved culture platform, hESC-CMs align and display the typical banding pattern consistent with organized sarcomeric structure patterns[59] (Figure 5) that is never seen in controls. Quantitative assessment based on nucleus shape and actin organization show that the hESC-CMs exhibit increased alignment on the microgrooved

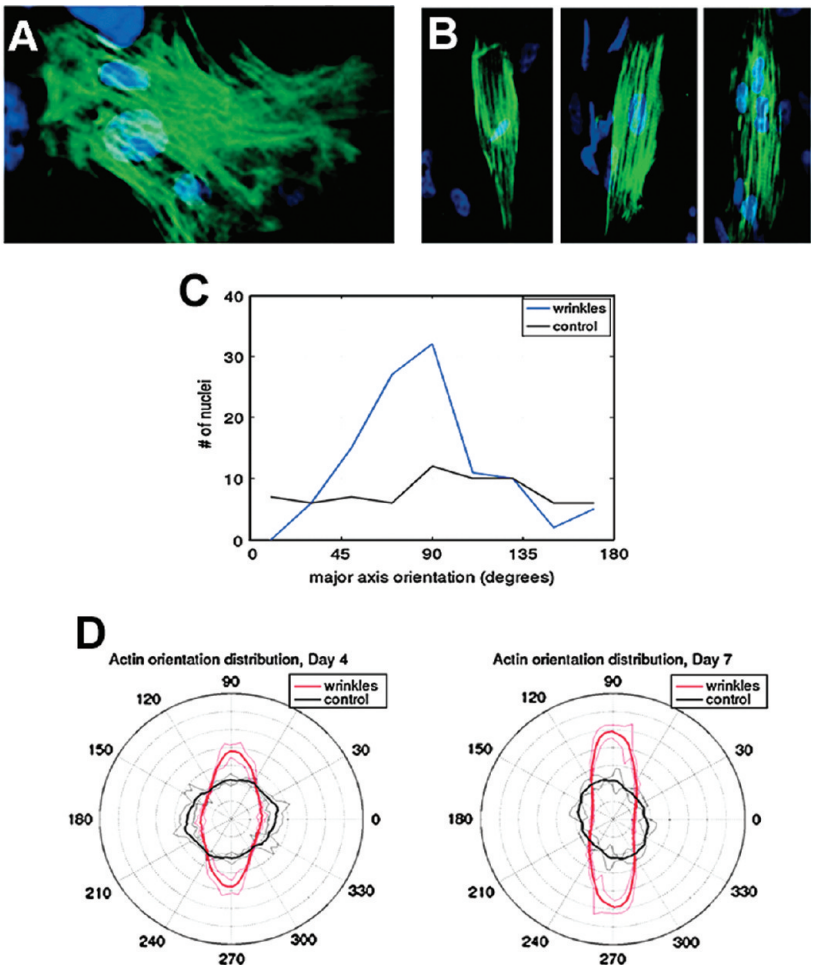

Figure 5. Confocal micrographs of hESC-derived CMs alignment on wrinkles. Human ESC-CMs were isolated and cultured on flat substrate (A) and wrinkle substrates (B) for 8 days. Green indicates tropomyosin staining, blue nuclear staining DAPI. (C) Image processing was used to detect the orientation of the DAPI-labeled nuclei. (D) Anisotropy analysis of control (black) versus red (on wrinkles) showing that 90° is direction of wrinkles. The thinner lines indicate the standard deviations. Reprinted with permission from ref 59. Copyright 2010 Mary Ann Liebert, Inc.

substrates. Functionally, high-resolution optical mapping reveals that aligned monolayers of hESC-VCMs display anisotropic conduction properties with distinct longitudinal and transverse velocities, a signature characteristic of the native heart, not seen in control randomly organized monolayers (Lieu, Wang, Khine and Li, unpublished data). This finding resembles those previously reported for neonatal rat ventricular cardiomyocytes.[67] Three-dimensionally, the Costa lab constructs engineered cardiac tissue constructs (ECTs) including cardiac papillary-like muscle strips as well as ventricle-like "organoid" chambers that exhibit key characteristics of cardiac physiology by ejecting fluid and displaying force−frequency and pressure−volume relationships.[68,69] Various similar tissue engineering approaches have been independently developed and employed by several other laboratories.[70−74] Although these techniques were often first developed using neonatal rodent ventricular cells, they are now being applied to hESC/iPSC-CMs with improved cell yields from directed cardiac differentiation. Not only will further optimization of hESC/iPSC-based ECTs provide powerful tools for disease modeling, drug/cardiotoxicity screening and clinical translations, but the physiologic 3D environment constructed also promises to reveal novel insights not possible with conventional rigid 2D culture systems.

■ MICRORNA- AND PLURIPOTENT STEM CELL-BASED HEART THERAPIES

MicroRNAs (miRs) are nonencoding RNAs of ∼22 nucleotides that function as negative transcriptional regulators via degradation or inhibition by RNA interference.[75,76] They have been suggested to regulate ∼30% of human genes.[77] To date, 706 human miRs have been identified, of which approximately 50% of pre-miR sequences are located within introns according to the Sanger Database. The expression of miRs is highly regulated and dependent on tissue, cell type, metabolic status and diseases states. Recent studies have demonstrated that miRs are important regulators of cardiovascular cell differentiation, growth, proliferation and apoptosis. In the heart, some of the most abundant miRs include miR-1, let-7, miR-133, miR-126-3p, miR-30c, and miR-26a.[78] In particular, miR-1, miR-133[79] and miR-208 are specific to cardiac and skeletal muscles. Various recent profiling efforts have revealed profound alterations of miR expression in the pathogenesis of human heart failure.[80−82] Abnormal miR expression patterns have also been detected in hypertrophy and arrhythmias.

There have been few studies on the role of miR in hESC/iPSC-CMs. Ivey et al. showed that miR-1 promotes differentiation of mouse and human ESCs into the cardiac lineage, as

evidenced by an increased expression of nkx2.5 and percentage of spontaneously contracting EB outgrowths.[83] Our group recently mapped and compared the miR profiles of hESC, hESC-derived VCMs (hESC-VCMs), human fetal and adult VCMs.[84] Sixty-three miRNAs were differentially expressed in hESC-VCMs compared to hESCs, of which 23 were also expressed highly in human fetal and adult VCMs. miR-1 and miR-499 displayed the highest degree of differential expression and thus were chosen for further characterization. Overexpression of miR-499 by lentiviral transduction significantly enhanced the differentiation of cardiac progenitors into VCMs. By contrast, miR-1 overexpression did not affect the yield of VCMs from hESC differentiation, but decreased action potential duration (APD) and hyperpolarized resting memebrane potential (RMP)/maximum diastolic potential (MDP) in hESC-VCM due to increased I_{to}, I_{Ks} and I_{Kr}, and decreased I_f as signs of maturation (unpublished). Taken collectively, miR-499 seems to promote ventricular specification of hESCs, while miR-1 serves to facilitate electrophysiological maturation.

HUMAN ESC-BASED BIOARTIFICIAL SAN (BIO-SAN) AS AN ALTERNATIVE TO ELECTRONIC PACEMAKERS

Normal rhythms originate in the SAN, whose malfunction leads to rhythm generation disorders. While conventional treatments of pharmacological intervention and/or implantation of electronic pacemakers are effective, they are associated with shortcomings such as potential infection, finite battery life, permanent implantation of leads, and the lack of intrinsic responsiveness to neurohumoral regulation. Various cell- and gene-based approaches have been developed by us[28,51,55,85] and others[86,87] to reconstruct a functional bioartificial SAN (bio-SAN) as an alternative or supplement to electronic devices. By genetically overexpressing a genuine pacemaker gene product, the hyperpolarization-activated cyclic nucleotide-modulated (HCN) channels, we confer upon normally quiescent cardiac muscle cells the ability to intrinsically fire APs similar to genuine nodal pacemaker cells.[28,51,55] Importantly, when this HCN gene-based bio-SAN is tested in a large animal (swine) model of sick sinus syndrome (SSS),[85] side-by-side comparison shows that bio-SAN significantly reduces the dependence on device-supported pacing by electronic pacemaker from ~85% to ~15% in the same animal after receiving our treatment. Alternatively, pluripotent human embryonic stem cells (hESCs) can be differentiated into electrically active cardiac derivatives that mimic pacemaker cells for transplantation to construct an *in vivo* bio-SAN.[28,87] Unlike myocardial repair, cell-based bio-SAN requires relatively many fewer cells (several thousands versus tens or hundreds of millions or more). Furthermore, a simple injection of cells (or gene delivery vehicle) suffices to induce a local ectopic pacing site. As such, a functional bio-SAN, with its structure as a node or sphere of cells, is comparatively less complex than the thick left ventricular wall. We are currently exploring the possibility of using nodal progenitors. We are also testing the long-term safety and efficacy of bio-SAN and noninvasive catheter-based delivery techniques for implantation. The use of iPSC as bio-SAN has not been explored.

MYOCARDIAL TRANSPLANTATION

Myocardial infarction (MI), generally known as heart attack, results from the blockage of blood supply to the myocardial tissues leading to cardiac cell death. Delayed thrombolytic treatment of the blocked coronary artery leads to irreversible and permanent impairment of the heart. Sudden cardiac death (SCD) due to ventricular arrhythmias following MI-induced impairment remains a leading cause of morbidity and mortality in the industrialized world. Even if a patient survives an attack, the remaining CMs hypertrophy in an attempt to meet the functional demands, while cardiac fibroblasts secrete collagen and other extracellular matrix proteins during scar formation, which may further impair ventricular function. Since terminally differentiated myocardial tissue possesses limited regenerative capacity, clinical treatments remain palliative. Pluripotent stem cells have led to the development of various cell-based therapies that have shown significant promise in at least partially restoring cardiac function.[9] However, as already described, hESC- and iPSC-CMs display a range of functional and structural properties that are remarkably similar to those of immature or failing heart cells. Generation of functionally mature myocytes which can activate complex signaling processes that reverse remodel the failing heart and prevent the incidence of arrhythmias is an area of active investigations. Transplantation of hESC-CMs intramyocardially into immunocompromised (NOD-SCID) mice with MI resulted in rapid formation of grafts with significant improvement in cardiac function at 1 month,[88] similar to those observed for rats with MI.[30] However, this beneficial effect does not sustain after 12 weeks despite graft survival and irrespective of its size,[89] suggesting that the long-term efficacy of cardiac cell transplantation is uncertain. Indeed, the same beneficial effect of hESC-CMs transplantation observed in acute MI models is not seen in chronic MI models.[90] Better delivery protocols and engineered constructs may be needed to overcome these hurdles.

SUMMARY

The availability of hESC and iPSC and their successful differentiation into genuine human heart cells have enabled clinicians and scientists to gain insights into the early development of the human heart as well as to pursue to the revolutionary paradigm of heart regeneration. However, hESC- and iPSC-derived CMs appear to be both structurally and functionally immature. One of the most important factors to consider before any potential benefits of hESC-CMs are clinically assessed would be to ensure their safety. Furthermore, the successful use of derived CMs as human heart disease models and cardiotoxicity screening tools depends on their ability to recapitulate the properties of their adult counterparts. Further studies are required to promote their maturation. When combined with other advances in driven differentiation and cardiovascular progenitor identification,[34,91,92] the approaches can facilitate the translation of hESC/iPSCs into clinical and other applications.

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